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Effect of age on the maturation of rat-skin collagen

The purpose of this study was to assess the effect of age on the solubility of rat-skin collagen, on the mechanism of its maturation to insoluble forms and on the turnover of the different collagen fractions.

The solubility of rat-skin collagen varies in the different reports from 0.05 to 89%[1-4]. Similar wide range exists in the observed turnover rates[1,5,6]. The contradictory results arise partly from the poor definitions of the fractions[4] but also as the effect of varying age.

L-[^{3}H]Proline (The Radiochemical Centre, Amersham, England) was injected intraperitoneally into rats of different ages (1 μC/g). After 4 h the rats were killed, and 0.45 M NaCl-soluble and 0.5 M acetic acid-soluble collagen fractions were isolated from the skins by repeated extractions until the protein content of the supernatants was negligible. The residue was regarded as insoluble collagen. All the fractions were dialyzed exhaustively against water, lyophilized and analyzed for hydroxyproline[7] and hexosamine[8]. The specific activity of hydroxyproline was determined according to JUVA AND PROCKOP[9] with Tri-Carb 3214 liquid scintillation spectrometer (Packard Instrument Co.). A method was developed for the measurement of the specific radioactivities of the collagen components in starch-gel electrophoretic fractions[10].

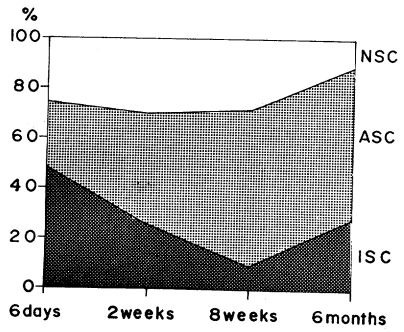

Fig. 1. Effect of age on the distribution of the solubility fractions of rat-skin collagen. NSC, neutral salt-soluble collagen; ASC, acetic acid-soluble collagen; ISC, insoluble collagen.

The skin of newborn or 6-day-old rat is characterized by a proportional abundance of insoluble collagen (Fig. 1). During the period of rapid growth there accumulates mainly acid-soluble collagen. The insoluble collagen is heterogeneous yielding upon heating for 30 min at 40° a fraction designated ISC_{40}, which increases with advancing age (Table I). This ISC_{40} fraction contains proportionally more of the large aggregates than the soluble collagen fraction[4]. Also the ratio hexosamine to hydroxyproline decreases rapidly in the insoluble fraction with age. These data suggest that in the

TABLE I

EFFECT OF AGE ON THE COMPOSITION OF INSOLUBLE COLLAGEN OF RAT SKIN

ISC, insoluble collagen; ISC_{40}, fraction of insoluble collagen which dissolves on heating in 0.5 M acetic acid at 40° for 30 min.

Age	*ISC_{40} of total ISC (%)*	*Ratio of hexosamine to hydroxyproline (w/w) in ISC*
6 days	7.1	0.46
8 weeks	29.1	0.07
6 months	44.0	0.02

young animals the building of insoluble skin collagen occurs through the interaction of collagen with glycosaminoglycans, which decrease towards the advanced age, when the interaction of the collagen components may be the predominant reason for the insolubility. About a half of this cross-linked "insoluble" collagen of adult rats dissolves, however, on mild heating and intact components are liberated.

Table II shows the distribution of [^{3}H]hydroxyproline between the collagen fractions. During the incorporation period of 4 h the label has advanced into the acid-soluble and insoluble fractions faster in young than in adult rats. The half-life of neutral salt-soluble fraction of young rats (23 h) was several times shorter than that of the adults (74 h). This difference cannot be explained by the varying growth rates. We conclude from the evidence given in Fig. 1 and Tables I, II that the maturation of collagen is faster in young than in adult rats and that the composition of insoluble collagen varies with the age.

To investigate the metabolism of the collagen components in the soluble fraction of rat skin, L-[^{3}H]proline was injected into 6-day-old rats (1 μC/g), but the rats were killed after the incorporation periods of 0.5, 1, 4, 24 and 85 h. Soluble collagen was extracted with 0.1 M acetic acid, purified and fractionated to components as described earlier[4]. In the α_2 component the label appears and disappears faster than in the α_1 component (Fig. 2). The specific activities of the β and γ components rise slower indicating a precursor–product relationship with the α components. The simultaneous

TABLE II

EFFECT OF AGE ON THE INCORPORATION OF PROLINE INTO COLLAGEN FRACTIONS OF RAT SKIN

NSC, neutral salt-soluble collagen; ASC, acetic acid-soluble collagen; ISC, insoluble collagen. The figures are means of 3 determinations from samples pooled from the indicated number of animals. The ratios are calculated from the specific activities of hydroxyproline.

Age	*Number of rats*	*Hydroxyproline (counts/min per g fresh skin)*	*NSC / ASC*	*NSC / ISC*	*ASC / ISC*
6 days	8	17 560	25.2	11.8	0.47
8 weeks	4	11 440	59.3	79.0	0.90
6 months	3	8 860	257.0	565.0	1.26

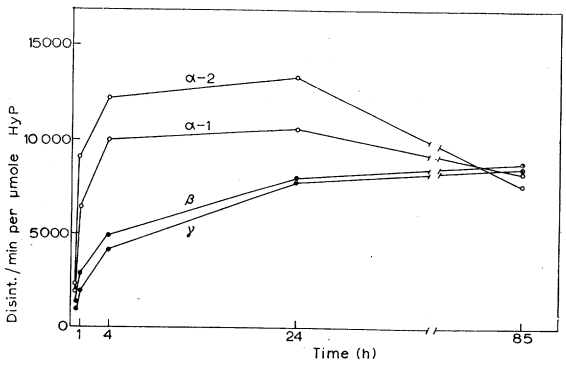

Fig. 2. Specific radioactivities of the α_1, α_2, β and γ components in the 0.1 M acetic acid-soluble collagen fraction of rat skin. Each point represents the mean of 4 determinations. When the points at 1, 4 and 24 h were considered, the difference between the α_2 and α_1 components was statistically significant both by the t-test ($t = 8.25$, $n = 22$, $P < 0.001$) and by the analysis of variance ($F = 22.3$, $f_1 = 1$, $f_2 = 18$, $P < 0.0005$).

labelling of the β and γ components suggests that they are formed from the single-chain monomers at the same time. In the analogous experiments on adult rats with labelled glycine[11] the specific activities of the α components were higher than those of β components after 7 days and even after 144 days[12]. Thus at the component level also the metabolism of collagen is faster in younger animals.

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